Physiological traits for crop yield improvement in low N and P environments

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Abstract

Nitrogen and phosphorus are recognized as essential elements in crop production, but the full extent of the requirement for these elements in the physiological processes leading to crop growth seems not to be always fully appreciated. Virtually all the biochemical compounds in plants that support development and growth contain N and/or P. Deficiencies in either element lead to a lost ability for plant growth such that there is a quantitative relationship between crop yield and accumulation by plants of each of these elements. Few options appear to exist to greatly diminish the requirement for either element in crop growth and the formation of seed yield. Consequently, crop yields cannot be increased without increased acquisition of N and P by plants. If the soil environment does not offer these elements, then crop yield will necessarily be restricted. While little opportunity exists to increase N recovery under low nutrient environments, several options can be investigated for increasing P accumulation by the crop. Ultimately, however, the rigid limitation on yields of inadequate N means that without external supplies of N for the cropping system, biological fixation of N must be enhanced to increase N input. In particular, it appears that considerable research needs to be focused on whole-plant processes in legumes that lead to enhanced symbiotic N fixation. A critical aspect of increased legume production will be improved management of P to allow legumes to achieve high N fixation rates and yields.

Introduction

Nitrogen and phosphorus have long been regarded as essential elements that must be provided to crops to obtain good growth. However, the constraining nature of the 'essential' status of these elements on the physiology of crop growth is sometimes overlooked, particularly when issues relative to increasing crop yields are examined. In this paper, we examine a series of questions that help to define the role of N and P in crop growth. What is the physiological role of each of these elements? Can the need for N and P be decreased in the plant? Can N and P allocation to the grain be increased to improve yields? What is the quantitative yield limitation imposed by N and P?

Two additional questions related to the physiology of crop plants are discussed in regards to increasing the acquisition of N and P. Can crop N and P uptake from the soil be increased by altering physiological traits of the plant? What is the role of legumes in improving N levels in low-nutrient environments? In the discussion of each of these questions, the intention is to give some perspectives about the opportunities for physiological improvement of crops that might result in increased yields in low-nutrient environments.

What are the essential physiological roles of N and P in crop growth?

Nitrogen and phosphorus are both integral components of virtually all the biochemical compounds that make plant life possible, as discussed below. There

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is no conceivable alternative to these two elements in constructing the biochemical machinery of plants. It is absolutely clear that both N and P are essential elements in their structural, biochemical and physiological roles contributing to crop growth.

Furthermore, the dependence of crop growth on N and P is a quantitative one. Virtually all of the compounds that participate in metabolism, cell growth and tissue development contain N and/or P. Therefore, diminished levels of N and P have many ramifications that result in quantitative decreases in the rate of growth, and ultimately yield. Commonly, decreases in either N or P, which is likely under low-nutrient conditions, leads directly to decreases in the levels of the critical compounds required to sustain high rates of growth. It should be no surprise, therefore, that there is frequently a close correlation between crop yield and the amount of N and P that the plants have accumulated.

Nitrogen

Nitrogen is an essential component of amino acids and nucleic acids. Consequently, without N it is not possible to synthesize the necessary proteins, enzymes, DNA and RNA required in virtually all plant cells for their initial development, sustained growth and functioning to support other tissues of the plant. Deficiencies in reduced N necessarily results in less biochemical machinery to catalyze plant metabolism and to generate new cells. Consequently, nitrogen deficiencies result in decreased crop leaf area, photosynthetic assimilation and seed growth (Sinclair, 1990).

The biochemical process in the plant that has quantitatively the greatest demand for N is photosynthesis. In fact, RuBisCO, which is the enzyme that initially captures CO_2, accounts for over half the protein in leaves of C_3 plants. In C_4 species, RuBisCO may account for a quarter of the protein in the leaves and additional enzymes of the organic acid cycle for CO_2 capture add to the dependence of photosynthesis on N content in the leaves. Therefore, declines in N content in leaves of all crop species are invariably associated with declines in leaf photosynthetic rates. A close correlation between maximum leaf CO_2 assimilation rate and N per unit leaf area has now be documented in a number of crop species including maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) (Muchow and Sinclair, 1994), sunflower (*Helianthus annuus* L.) (Gimenez et al., 1994), rice (Peng et al., 1995), potato (*Solanum tuberosum* L.) (Vos
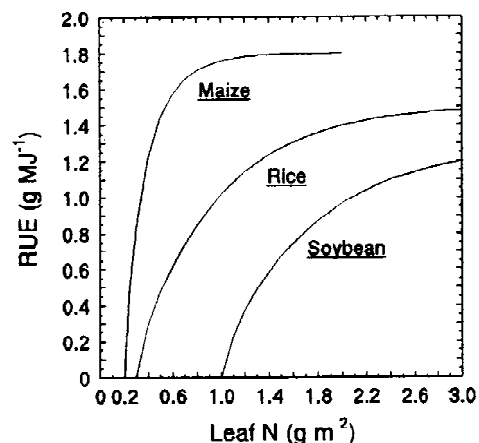

Figure 1. Theoretical relationship between radiation use efficiency (RUE) and average canopy leaf nitrogen content per unit leaf area for maize, rice and soybean (Sinclair and Horie, 1989).

and Van Der Putten, 1998 and sugarcane (*Saccharum* spp.) (Meinzer and Zhu, 1998). Indeed, much of the variation in leaf photosynthetic capacity for different cultivars, age of leaves and growth conditions can be attributed directly to differences in leaf N content (Sinclair and Horie, 1989).

A recent report by Meinzer and Zhu (1998) indicated that an additional consequence of the loss of activity in the photosynthetic enzymes is a decrease in apparent quantum efficiency. They found in sugarcane that the quantum yield of CO_2 assimilation is decreased linearly with decreasing N content. They attributed the decrease in quantum yield to a decline in the activity of the enzymes of the C_3 cycle. There is also the possibility that N deficiency in the leaves may result in increased photoinhibition of photosynthesis (Henley et al., 1991).

Crop radiation use efficiency (RUE, ratio of accumulated crop mass divided by cumulative intercepted solar radiation) is directly dependent on maximum leaf photosynthesis rate (Hammer and Wright, 1994; Sinclair and Horie, 1989). Because photosynthetic activity is dependent on leaf N content, it follows that RUE is also closely linked to leaf N content. A number of experimental studies have demonstrated such a linkage (Sinclair and Muchow, 1999). Theoretical studies (Hammer and Wright, 1994; Sinclair and Horie, 1989) have also developed functional relationships between RUE and leaf N content for different crop species, as illustrated in Figure 1.

The requirement for N in the construction of enzymes and nucleic acids for new cells results in a particularly large influence on new cell development

under N deficiency. One of the obvious manifestations of this limitation is in the inhibition of leaf area development under low N conditions. For example, Muchow (1988) reported substantial decreases under deficit N conditions in the area of individual leaves of maize and sorghum plants even though the number of leaves was virtually unchanged. Similarly, Gimenez et al. (1994) found large decreases in sunflower leaf area with low nitrogen fertility, whereas the CO_2 assimilation capability was decreased only to a small extent. In potato, Vos and Van Der Putten (1998) reported decreases in the area of individual leaves with decreasing N fertility, but leaf photosynthetic rates were not different among N treatments. Consistent with this last report, the inability to generate new cells for expanding leaf area under N deficit is commonly more serious in limiting crop growth than the potential decrease in photosynthetic activity per unit leaf area.

Phosphorus

Phosphorus is an essential component of cell structures, mainly as nucleic acids and phospholipids. It is especially critical in establishing the enzymatic machinery in energy storage and transfer, which in many cases involves membrane processes. Not surprisingly, P deficiency results in a loss in cell integrity (Ratnayake et al., 1978). The bonding properties of P also make it crucial for metabolic processes that are nucleotide-based, e.g. ADP, NAD and NADP, because of its unique energy-transfer properties. A general consequence of P deficiency is a decrease in the energy charge of cells [(ATP + 0.5ADP)/(ATP+ADP+AMP)] (Duff et al., 1989; Sa and Israel, 1991).

Inorganic P (Pi) is essential because it is used as a 'currency' in the generation of high-energy phosphate compounds such as ATP from ADP, which is especially critical in respiration. According to Bieleski (1973), P found in DNA/RNA/lipid-P/nucleotide-P would be found in typical proportions of 0.2/2/1.5/1 and Pi usually represents about 2/3 of total plant P. With the development of P deficiency, it is primarily Pi that decreases in plant tissue, prior to a decrease in adenylate-P (Rufty et al., 1993).

The concentration of Pi in plant cell cytoplasm of P-sufficient plants is commonly in the range of 5–10 mM (Bieleski, 1973), which is assumed to be necessary to sustain optimal activity (Foyer and Spencer, 1986). Rebeille et al. (1984) reported that mitochondrial respiration of sycamore cells (*Acer pseudoplatanus* L.) was not affected at a Pi concentration as low as 3 mm. Maintenance of a stable cytoplasmic Pi concentration appears to be essential for many enzyme reactions (Schachtman et al., 1998) to sustain optimal activity. This requirement, however, is met by using P stored in the cell vacuole to buffer cytoplasmic P concentration (Lee and Ratcliffe, 1993; Mimura et al., 1990).

Phosphorus distribution within plant cells is regulated so as to maintain the cytoplasm P concentration at a fairly constant level and vacuole P concentration varies to accommodate surpluses or deficiencies that may develop in cells (Lauer et al., 1989; Lee et al., 1990). Concentrations of P in the vacuole can vary from near zero under P-deficit conditions to up to 25 mm under non-limiting conditions. Consequently, physiological activity of a tissue has been observed to be fairly stable over a wide range in bulk tissue P concentration as long as vacuoles are able to buffer changes in P so that the cytoplasm P concentration remains at adequate levels. This buffering capacity makes it difficult sometimes to resolve the sequence of physiological effects of P deficiency, unless cytoplasm Pi concentration is determined apart from bulk tissue P.

One manifestation of the ability to stabilize cytoplasmic P levels is that photosynthesis rates are stable over a wide range of decreasing bulk leaf P. For example, in maize, several studies have shown a relative insensitivity of leaf photosynthesis to decreasing P (Khamis et al., 1990; Usuda and Shimogawara, 1991). Starch levels have actually been observed to increase in P deficit leaves (Fredeen et al., 1989, 1990; Lauer et al., 1989). Mollier and Pellerin (1999), who observed in maize that leaf area development was the first and most dramatic influence of P deprivation, found that RUE decreased only at a late stage in the low-P treatment of their experiment.

Developing cells do not have mature vacuoles, and as a result, do not have a large stored reserve of P to buffer growth under P-deficient conditions. Therefore, developing cells that require P for synthesis and maintenance of membrane integrity are especially vulnerable when the P supply is low. Leaf area development, as an example, decreases drastically under P deficiency in sugar beet (*Beta vulgaris* L.) (Rao and Terry, 1989) and in soybean (Rao et al., 1993). Fredeen et al. (1989) also reported for soybean that the first and most dramatic response to decreased P was decreased expansion of individual leaves.

In contrast to the sensitivity of leaf development to P deficiency, root development under low P is sus-

tained (Anghinoni and Barber, 1980; Cakmak et al., 1994) or even stimulated (Bates and Lynch, 1996; Rufty et al., 1993). As a consequence, the root–shoot ratio is increased under low P conditions relative to high P conditions (Freeden et al., 1989; Yan et al., 1996). This response in root growth has been hypothesized to result from the fact that shoot development is readily decreased by P deficiency so that additional carbon is allocated to the roots (Mollier and Pellerin, 1999). This hypothesis is consistent with observed increases in root carbohydrates levels (Cakmak et al., 1994) under P-deficient conditions.

Inorganic P is directly involved in enzymatic processes that require phosphorylation of carbon compounds such as during glycolysis (Duff et al., 1994) or during the dark phase of photosynthesis. Photosynthetic metabolism under P deficiency can be dramatically slowed because of a decreased rate of RUBP regeneration and a slower activity of the Calvin cycle enzymes (Fredeen et al., 1989, 1990). Although the photosynthetic response commonly occurs later than the reduction of leaf area upon P deprivation, it is of importance for plants growing under constant P deficiency.

Studies have shown that one of the major consequences of inadequate P is a decrease in the energy metabolism of the plant. A central reason for this response is the decrease of an adequate Pi supply to the mitochondria for the phosphorylation of ADP in ATP. For example, Rufty et al. (1993) found that low P in soybean (*Glycine max* L. Merr.) resulted in decreased [ATP] in roots, and this was associated with depressed nitrate uptake and transport by the roots. Sa and Israel (1991) reported that low levels of P resulted in low levels of ATP and energy charge in the plant-cell fraction of soybean nodules. In their study, the negative influence of low P availability on energy metabolism resulted in greatly decreased nitrogen fixation activity.

Can the need for N and P in plant growth be decreased?

Nitrogen

The plot of RUE against leaf N content (Figure 1) leads to an interesting possibility for differences in growth potential among species under N-limited conditions. In situations where the N availability only allows low leaf N content, there is a clear distinction among species. C$_4$ species as represented by maize in Figure 1 have the potential to sustain high values of RUE, while in other species, RUE, and consequently growth, falls to very low levels. For example, consider the case in Figure 1 where the N availability allows crop leaf N content to be only about 0.5 g N m^{-2} leaf area. Such a low leaf N is insufficient to even sustain soybean growth and rice is relegated to a very low RUE of only about 0.5 g MJ^{-1}. In contrast, maize is predicted to be able to sustain a RUE of 1.5 g MJ^{-1} even with this assumed low leaf N content. In this example, C$_4$ photosynthesis confers about a three-fold advantage to maize over rice for RUE. However, as discussed later, a high RUE in itself is not sufficient for high grain yield because the overall benefit of the C$_4$ trait for mass accumulation still must be matched with N and P acquisition to construct seed mass. Therefore, incorporation of the C$_4$ photosynthesis pathway into C$_3$ species may not bring any major benefit under low-N environments.

Moreover, it should be noted in Figure 1 that the tremendous advantage of the C$_4$ trait under low leaf N is diminished considerably when leaves of each species are allowed to reach N levels that result in maximal RUE for each species, and where incorporating a C$_4$ pathway in rice would confer a maximum RUE increase of only about 20%. That is, under conditions that give high leaf N for each species, RUE for maize is still greatest among the species at 1.7 g MJ^{-1}, but RUE in rice is about 1.4 g MJ^{-1} and soybean is 1.2 g MJ^{-1}. Therefore, under high fertility conditions, C$_4$ photosynthesis would be expected to confer as a maximum only about a 20% advantage in RUE for rice even if a fully functional C$_4$ system could be incorporated into its leaves.

Since a decrease in leaf area development is commonly the most substantial limitation on yield under low N fertility, options to decrease the sensitivity of leaf area development to N deficiency would be quite beneficial. One solution would be to produce thinner leaves, which presumably would require less N. That is, fewer cell numbers of the thinner leaves would decrease the requirement for N. The tradeoff, however, is that thinner leaves would likely lead to lower photosynthetic activity per unit leaf area. Sinclair and Horie (1989) presented calculations on crop mass production for various assumed levels of leaf N per unit area. Their results showed that decreasing leaf N per unit area, i.e. assuming thinner leaves, allowed leaf area to be greater but this advantage was almost completely offset by the lower photosynthetic activity per unit leaf area.

Another possibility for sustaining high mass accumulation under low N is to concentrate the available N into the leaves at the top of the leaf canopy. The theoretical analyses of Sinclair and Shiraiwa (1993) and Hammer and Wright (1994) showed a slight advantage in RUE for a decreasing gradient in leaf N content from the top to bottom of the crop canopy as compared to a canopy with no gradient. The analysis of Sinclair and Shiraiwa (1993) indicated that a canopy with a N gradient was more advantageous under conditions of low N accumulation than when N accumulation is high. The potential benefit of attempting to incorporate a N-gradient trait for the crop canopy is likely to be minimum, however, because such gradients have been found to already exist in crops even when supplied with adequate N (Pearcy and Seemann, 1990; Shiraiwa and Sinclair, 1993).

An interesting aside is the possible interaction of N storage in leaves and the trend towards developing crops with erect leaf angles. Sinclair and Sheehy (1999) calculated that high-yielding crops required large amounts of N to be stored in the leaves before seed growth. They speculated that to retain the large leaf area necessary to store the N, it was necessary for all of the individual leaves to receive a minimum amount of light so that they could be sustained. Therefore, to allow leaves at the bottom of the canopy to receive a minimum amount of light, it was necessary for the leaves higher in the canopy to be displayed at an erect angle. Their conclusion was that the advantage of erect leaves in modern crop varieties was to a large extent based on the need to retain leaves and store N in the leaves. In low-nutrient environments, the amount of N to be stored is low so that there is little need for erect-leaf crops.

Indeed, in low-nutrient environments, it is quite likely that horizontal-leaf crops would be more desirable. As discussed previously, loss of leaf area is one of the main consequences of inadequate N and P. To maximize light interception when only a low leaf area is produced, it is advantageous to display leaves in a more horizontal orientation. Furthermore, weed competition can be particularly troublesome under low-nutrient conditions. Crops with more horizontal leaves are more likely to shade competing weeds and lessen the negative impact of these weeds. Hence, crops to be grown in low-nutrient environments may well require a plant architecture that is markedly different from those being produced for nutrient-rich environments.

Phosphorus

The central role of P in energy transfer processes seemingly allows for few alternatives to decrease the requirement for P. There is evidence for alternate enzymes in the glycolysis pathway that minimize the requirement for P in respiration. Lambers (1982) reviewed evidence for the existence of a cyanide-resistant non-phosphorylating pathway of respiration in roots, which could be used as an energy overflow. More recently, Rychter et al. (1992) showed that the activity of this pathway was enhanced in mitochondria extracted from P- deficient bean roots. Duff et al. (1989) offered crucial evidence of enhanced activity of Pi-independent enzymes in the glycolytic pathway under low-P conditions. While these enzymes are less efficient energetically in glycolysis than the P-dependent enzymes, they offer a pathway for continued glycolysis under low Pi conditions (Theodorou and Plaxton, 1993). However, these intracellular enzymes showed a dramatic increase only after induction by severe P deficiency and with a lag compared to the increase in Pi influx capacity (Lefebvre et al., 1990). As of yet, there is no evidence whether this alternate glycolysis pathway is readily induced in crop species and whether there is genotypic variation for this trait under low P conditions.

There appears to be little opportunity to overcome the P requirement in the development of cell components, although internal P requirements seem to vary between and within species (Föhse et al., 1988). It is also striking that *Imperata cylindrica*, a challenging weed in deforested tropical soils (Hairiah et al., 1993) is able to thrive in low P soil with a shoot P concentration as low as 0.05%, four times less than shoot P concentration in bean or soybean. There are also reports showing genotypic differences exist in plant P concentrations. For example, Vadez et al. (1999) reported such differences among 48 genotypes of common bean. Similar to N, however, a decreased concentration of P in the vegetative tissues may not overcome the obligate requirement for P in the developing seed.

Can the allocation of N and P to grain be improved to achieve increased grain yield?

Both N and P are highly mobile in the plant. As a result, N and P that have been used in the development of the vegetative tissues of the plant can be transferred to the developing seed. The success in providing the

accumulated N and P to the seed is indicated by the harvest index for each of the elements. That is, the ratio of the amount of the element in the grain relative to the amount of the element in the total above-ground portions of the plant indicates the success with which the element has been transferred to the grain.

The values of nitrogen harvest index (NHI) are consistently found to be high in modern crops. It is not uncommon for NHI to be 0.75 or more. Teyker et al. (1989) selected maize populations based on seedling nitrate uptake, but NHI remained essentially unchanged at an average of 0.74 across five populations. In soybean, Crafts-Brandner (1992) reported NHI subjected to various P treatments to be stable in the range of 0.79–0.86. Rice NHI has also been reported to be stable across cultivars and environments, although the values are somewhat lower than achieved in other crops. The studies of Tirol-Padre et al. (1996) and Singh et al. (1998) with rice resulted in NHI values generally in the range of 0.62–0.73. Consequently, it may be possible to yet increase NHI of rice to some extent.

Phosphorus is generally present as Pi or in mobile compounds so that P is readily available for transfer to developing seeds. As a consequence, phosphorus harvest index (PHI) is commonly quite high. Crafts-Brandner (1992) reported PHI greater than 0.8 for soybean when grown under low P. Values of about 0.8 for PHI were reported for 12 genotypes of common bean studied by Yan et al. (1995b), similar to that found in the bean cultivar Calima (Snapp and Lynch, 1996). In cases when PHI is at 0.8 or greater, it seems unlikely that the fraction of P that is allocated to the grain can be increased substantially.

What is the quantitative yield limitation based on N and P accumulation?

Sinclair (1999) recently employed a phenomenological equation to estimate the maximum yield that might be expected based on accumulated N.

$$Y < N_{up} \cdot NHI/G_N, \tag{1}$$

where Y = mass of grain yield, N_{up} is the total N uptake by the crop, and G_N is the N fraction of the seed.

The basis of this equation is that Y can be no greater than N available to the grain ($N_{up} \cdot NHI$) to produce the required fraction of protein in the developing seed. Maximum crop yield can be calculated using Equation (1) as a function of the total N uptake by the crop.
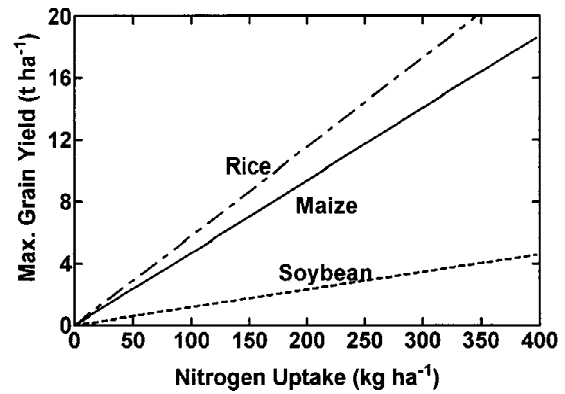

Figure 2. Calculated limits for grain yield of rice, maize and soybean at different levels of N accumulation by the crop. The nitrogen harvest index in each case was assumed to be 0.75 and the N content of the grain was assumed to be 13, 16 and 65 mg N g^{-1} for rice, maize and soybean, respectively.

As discussed previously, NHI is already high in many crops and it appears unlikely that this limit can be substantially improved.

One option to increase yield based on Equation (1) is to decrease G_N. A difficulty in decreasing G_N is that the food or feed value of the grain of many crop species is determined to a large extent by the value of G_N. Decreasing the protein content of rice or wheat, for example, will dramatically influence their nutritional value. Furthermore, the value of G_N tends to be stable. For example, Tirol-Padre et al. (1996) compared the grain N content of 180 rice genotypes of differing maturity dates and in two years of low fertility conditions. They found across all maturity dates a mean of about 10.5 mg N g^{-1}, and the CV within maturity group was less than 5%. Similarly, Singh et al. (1998) found in a comparison of 20 rice cultivars that grain N under low N fertility ranged from 8 to 10.5 mg N g^{-1}.

Equation (1) indicates that ultimately crop yield is directly limited by N_{up}, the amount of N accumulated through the growing season. Crop yield can be no greater than that allowed by N_{up} regardless of improvements in the accumulation of crop mass or in the biochemical pathways. This limitation can be shown graphically by plotting Y as a function of N_{up} as done in Figure 2. In these calculations, NHI was assumed to be 0.75 and G_N was assumed to be 65 mg N g^{-1} for soybean, 16 mg N g^{-1} for maize, and 13 mg N g^{-1} for rice.

It is clear in Figure 2 that in those nutrient-limited environments where N_{up} is low, the maximum yield is necessarily low. In those environments where a crop can accumulate no more than about 50 kg ha^{-1}, max-

imum yield is approximately 2 t ha^{-1}. Such levels of N_{up} and yield were essentially the limits of good agricultural practices (rotation, manure, etc.) before the advent of the use of chemical fertilizers (McCloud, 1998). Of course, a yield increase to 2 t ha^{-1} may be welcomed in many low-nutrient regions and the use of good, traditional management practices may offer sufficient nutrient to reach these yield levels. An important alternative indicated in Figure 2, however, is the use of a legume crop such as soybean. Soybean, which can obtain N from symbiotic fixation of atmospheric N_2, may be able to accumulate N well above 50 kg ha^{-1} and as a result achieve yields above the 2 t ha^{-1} limit in nutrient-limited environments without the addition of chemical N.

The approach presented in Equation (1) can also be used to define maximum crop yield based on the accumulation of P. To do this analysis for P, good information is needed on the level of P required to ensure unrestricted seed growth. These data, however, do not appear to be readily documented across a number of crop species. Data of P concentration in soybean were measured in eight soybean cultivars and found to vary from 5.7 to 7.1 mg P g^{-1} (Hanway and Weber, 1971a). Variation in seed P concentration was reported by Yan et al. (1995a) among 12 cultivars of common bean to be larger than soybean with a range of 2.7–5.0 mg P g^{-1}. Certainly, more of these types of data are required and an important research topic for the future might well be to understand the P levels that are required in seeds, as there seems to be larger variations in G_P than in G_N. Such data could lead to an analysis similar to that offered by Equation (1) of establishing maximum yield limits based on accumulated P (P_{up}).

Of course, some caution is needed in discussing independently the influence of N and P on the limits to crop yield. There is certainly interaction in the nutrient use by plants and the influence of this interaction on crop yield can be complex (Sinclair and Park, 1993). For example, Hanway and Weber (1971b) observed in soybean that seed N concentration was fairly constant, while seed P concentration varied to a much greater extent and was dependent on N supply. Therefore, if seed yield is closely dependent on N_{up} it might not be so closely dependent on P_{up}, which gives some leeway for seed production under limited P_{up}.

Can N and P uptake from the soil be increased in low-nutrient environments?

Since N and P accumulation are absolutely essential in obtaining maximum crop yields, it should not be surprising that crop plants have developed nutrient uptake systems that are very effective at recovering available N and P in the soil either as a result of natural selection or breeder selection. Uptake systems in the roots have high affinities for each element and are operational at very low concentrations (Schachtman et al., 1998). If the element is available at the root surfaces and there is a physiological deficiency for the element in the plant, roots have the capacity for uptake rates that are many fold greater than observed under normal field conditions.

Nitrogen

There are at least two uptake systems in plant roots for nitrate that allow rapid uptake of N. The Km for nitrate uptake in the low-concentration system is about 20 μM (Hole et al., 1990; Siddiqi et al., 1990) so roots are able to withdraw N from the soil solution to very low levels. There is, however, considerable evidence that N inflow rates per unit root length in field-grown crops are substantially less than the maximum inflow measured under controlled conditions (Feil, 1997). For example, the capacity for nitrate uptake by wheat roots has been found to be many fold greater than that required to support plant growth at all stages of plant development (Oscarson et al., 1995). This conclusion is further supported by the very high rates of N_{up} by field-grown rice that were observed for limited time periods when N supply in the soil was high (Cassman et al., 1998; Peng and Cassman, 1998).

The fact that N_{up} is not sustained at very high rates by crops under conditions of high N availability indicates a strong feedback system that substantially throttles N uptake capacity (Imsande and Touraine, 1994). The accumulation of nitrate in plants has been shown to have a direct and rapid feedback on the uptake system (RajRaman et al., 1995). In addition, the growth of the plant appears to feedback to regulate N content to a fairly constant level under adequate N supply. For example, Singh et al. (1998) found that there was no statistical difference in the accumulation of N among a number of rice genotypes of the same maturity classification.

One consequence of this tightly regulated feedback appears to be the common observation of a close re-

lationship between %N and crop mass within a crop species when soil N availability is high (Lemaire and Gastal, 1997). Greenwood et al. (1990) proposed that a common relationship existed between %N and crop dry weight for those crops that have received N adequate to achieve maximum crop growth rates. This relationship defines a curve of the critical nitrogen concentration levels for each crop species, and observations below this curve identified conditions where crop growth was being limited by inadequate nutrient. While this approach does not lead to a physiological understanding of N use by the crop, it highlights the quantitative dependence of growth on accumulated nutrient. Sheehy et al. (1998), for example, used this approach to examine the limitation of N accumulation on rice growth.

Since the uptake capacity by roots for N is high, the total amount of available N in the soil defines the upper limit for N accumulation. Especially in low-nutrient environments, the challenge is to maximize the uptake of the available soil N. Nitrate is highly soluble and moves readily in the soil so that there is no need for an extraordinary root density to recover the N in the soil solution. Root length densities of only 1.0–1.5 cm cm^{-3} appear adequate to extract soil N. Greater root depth is of little benefit because the amount of N available at deep soil depths in nutrient-limited environments is usually quite small.

Increased N uptake from unfertilized soils is likely limited to maximizing the recovery of mineralized N from the soil. The simplest and most direct approach to obtaining more mineralized N is to extend the length of the growing season so that a growing crop is present over a longer time period to immediately take up the newly available N. Tirol-Padre et al. (1996) reported in a comparison of 180 rice cultivars that in a year of low nitrogen availability there was a high correlation ($r^2 = 0.81$) between grain yield and total N uptake. The variation in N uptake among the cultivars was, in turn, highly correlated with the duration of the season. Consequently, longer-season cultivars may be a useful alternative to increase N_{up} in low-N environments. The disadvantage of the long-season approach, however, is that the potential exposure of the crop to various stresses is increased.

Phosphorus

Similar to N, soluble P in the vicinity of roots is also readily recovered by plant roots. At least two uptake systems have been demonstrated for inorganic P, and the high-affinity system has a Km of 3 μM (Furihata et al., 1992; Schachtman et al., 1998). Consequently, P is recovered to low concentrations in the soil. This is consistent with the report of Crafts-Brandner (1992) that the amount of P accumulated by soybean was in direct proportion to the total amount that was available to the plants.

The low mobility of P in soils, however, is a serious limitation (Amijee et al., 1991). It is crucial that the P-absorbing surfaces in the soil are extensive and prolific to make contact with the available P. Clarkson (1985) concluded that P was only depleted in the rhizosphere to a distance of about 100–200 μM from the root surface. Lynch and Beebe (1995) concluded that it was essential to have "a vigorous, highly branched root system with many growing points" to maximize P uptake. Yan et al. (1995a) reported that those common bean genotypes that performed best on low P-available soils were those that produced the greatest root mass.

Probably more important than branched roots for P uptake is the presence of root hairs (Gahoonia and Nielsen, 1998). P depletion from the root surface at a distance that approximates the length of the root hairs (Clarkson, 1985) opens the possibility that longer root hairs would allow for an increase in P uptake (Itoh and Barber, 1983), while representing very little investment in carbon and P. Of course, a search for diversity in root hair length is likely to be an arduous and time-consuming effort. Such a search is made more difficult because root hair length varies with the P concentration to which the roots are exposed (Bates and Lynch, 1996).

Another approach to increase the P-absorbing surfaces is to take full advantage of the symbiosis formed between roots of some plant species and mycorrhiza. This topic has received considerable attention and has been reviewed by Smith and Gianinazzi-Pearson (1988). There appears to be a great challenge in managing mycorrhiza for annual crops such that the soil is adequately inoculated, plant infection and mycorrhiza development occur in a timely fashion, and a highly functional symbiosis develops. At this point, it appears that mycorrhiza may be of greatest benefit in cropping systems that include a perennial crop where a long time-period for the development of the symbiosis is possible.

In many cases, the essential requirement for increasing P uptake by a crop is a need to increase the availability of P in the soil. The concentration of the soluble fraction in the soil is usually low, seldom going above 5 μM (Bieleski, 1973; Clarkson and Grignon,

1991). The soluble fraction needs to be continuously replenished with P to avoid deficient conditions, which depends on an acceptable level of exchangeable P. An exchangeable P concentration of 15 ppm is considered as critical for bean production (Thung, 1991). The challenge, especially under inherently low P soils, is to sustain sufficient soluble and exchangeable P when P is present in a fixed form in the soil.

Indeed, much of the soil P is unavailable to plants because it can be fixed in the soil in both inorganic and organic forms. Hydroxide forms of Al, Fe or Ca account for much of the insoluble P in the soil. The solubility coefficients range from a low value for Ca–P to very low values for Al–P or Fe–P. The organic forms of P are present as live soil bacteria and as mostly inositol phosphate and phytate (Clarkson, 1985). There are options, however, to develop physiological traits of plants that will increase the transformation of P from its fixed form to forms that can be taken up by plant roots.

The availability of inorganic P is commonly enhanced under acidic conditions in the soil. Roots have the ability to release both organic acids and hydrogen ions from roots to acidify the surrounding soil so as to increase P availability (Lynch and Beebe, 1995). Differences have been identified among crop species in their ability to acidify soil and extract inorganic P. In an experiment on an unfertilized Alfisol soil with a high iron phosphate level, Ae et al. (1990) observed that pigeonpea thrived, while four other crop species died within 1 month after sowing. They found that pigeonpea was unique in that it acidified the soil by excreting piscidic acid, which solubilized $FePO_4$.

There also are indications that there may be genotypic differences within species for acidifying the soil and extracting P. Lynch and Beebe (1995) examined 364 genotypes of common bean and found substantial variation in yield when grown under P-deficient conditions, indicating a genetic potential for increasing P extraction. In comparing six contrasting common bean genotypes, Yan et al. (1996) did not find a difference in their ability to acquire P from aluminum phosphate, iron phosphate or inositol phosphate. One genotype, however, was superior in acidifying the rhizosphere and extracting P from CaHPO4.

Release of P from organic forms of P in the soil can be catalyzed by phosphatase enzymes. These enzymes either originate from exudates directly from plants or from microbes whose growth is stimulated by plant exudates. Goldstein et al. (1989) demonstrated increased protein secretion from tomato cells suspen-ded in P-deficient media and that this secretion was associated with enhanced acid phosphatase activity. Regardless of the source of the phosphatases, differences in activity have been reported in comparisons of plant cultivars. Helal (1990) reported differences among cultivars of common bean in root phosphatase activity that were associated with differences in plant P uptake when the plants were grown on a solution containing organic P (inositol hexa phosphate). Similarly, Asmar et al. (1995) found differences in barley genotypes in their ability to induce soil phosphatase activity. The level of induced phosphatase activity was correlated with the hydrolysis of organic P compounds within the root hair zone. However, the exact role of phosphatase is not yet totally clear, as the major organic P compounds in soil, inositol P and phytate can be poor substrates for phosphatases (Clarkson, 1985). Furthermore, it is unlikely that plants would successfully compete for organic P with soil micro-organisms which permeate the soil. A possible role for phosphatases might be to recover Pi from organic P lost from the plant in the immediate vicinity of the roots.

Considering the fact that much of the P taken up by the plant is dependent on the release of P from unavailable forms, one approach to increase overall crop uptake is to extend the duration of the growth so that it has a longer time frame to accumulate P. Unlike N, there is no direct evidence that a long season allows time for more P to be released in the soil for root uptake. However, there may be a possibility that a longer season with delayed podfill would result in increased P uptake because roots remain functional for P uptake until late pod-filling (Snapp and Lynch, 1996). Also, Yan et al. (1995b) reported that some of the highest yielding common beans under low P are the type III and IV, which are climbing types. No details were given on time of harvest for individual genotypes, but climbing types commonly have a longer season than bushy types.

What is the opportunity for legumes in low-nutrient environments?

High yields are simply not possible without the accumulation of substantial amounts of N and P in the crop. One option for obtaining N, at least without relying on a soil source, is to take full advantage of those crop species that can symbiotically fix atmospheric N_2. In this situation, some of the carbon fixed by the plant is used to provide the energy required to 'fuel' the reduc-

tion of atmospheric N_2 for incorporation into organic molecules. While this process is energetically expensive, it might be very effective in low-N environments where assimilated carbon is abundant relative to N.

Grain legumes seem especially well suited to contribute to increased crop production in low-nutrient environments because they can be used both to produce a seed crop themselves and to generate high N-content plant residue. Since plant residues from grain legumes are sometimes considered poor sources of N because large amounts of the N is exported to the grain, it might be important to consider low harvest index, high N_2-fixing legumes or non-grain legumes. Rotations that include a legume as a green manure crop would be especially beneficial in increasing soil N fertility. Also, perennial legumes, which might be used in an agroforestry cropping system, offer an opportunity for long-term N_2 fixation as well as a long growth cycle to recover P from the soil.

Considerable attention has been given to increasing N_2 fixation activity of legumes. Much of the research, especially with the advent of molecular genetics, has focused on improved bacteria. Except in those locations where the appropriate bacteria to establish the symbiosis did not exist, little progress has been made in increasing legume N_2 fixation activity using improved bacteria. The performance of bacteria in isolation, however, does not translate well to plant performance under field conditions. The regulation of N_2 fixation activity tends to be a whole-plant phenomenon and unless the bacteria is ineffective at fixing N_2 there is little regulation at the bacteria level. Furthermore, it has proved to be very difficult to introduce alien bacteria into a soil environment where bacteria capable of forming a symbiosis already exist.

Attempts to increase N_2 fixation activity by altering the host plant have also achieved only limited success. In this case, much of the effort has focused on selecting hosts that result in greater nodulation. Again, nitrogen fixation activity is related to whole-plant activity and greater nodulation does not necessarily lead to greater N_2 fixation. Pracht et al. (1994) incorporated genes into soybean lines that produced hypernodulation on the roots but this resulted in no increase in either N_2 fixation activity or yield. A similar attempt has been made in the breeding of the 'RIZ' lines of beans (Kipe-Nolt and Giller, 1993) with no great success. On the other hand, a comparison of widely divergent lines of chickpea showed greater nodulation capacity was associated with greater N_2 fixation and grain yield (Rupela et al., 1997).

Another major limitation for enhancing N_2 fixation, especially in grain legumes such as common bean (Hardarson, 1993), is the great sensitivity of N_2 fixation to small amounts of residual soil nitrogen. Hardarson et al. (1993), however, identified genotypic variability among common bean for N_2 fixation with residual soil N.

One important success for increase N_2 fixation activity has occurred in alfalfa (*Medicago sativa* L.). Selection for several traits associated with increased N_2 fixation and plant growth resulted in the selection of parental genotypes and subsequently the development of a cultivar with increased N_2 fixation activity (Barnes et al., 1988). Since this concentrated and focused effort on selection for N_2 fixation activity has not been attempted in other legume crops, such an approach may offer a good option for increasing N_2 fixation productivity in low-N environments.

A constraint on the development of legumes for low-nutrient environments is the sensitivity of N_2 fixation to stress conditions. For example, N_2 fixation in several legumes was found to be very sensitive to soil drying (Sinclair and Serraj, 1995). Since many low-nutrient environments impose several stresses on the crop, intensive research is likely needed to incorporate a number of traits to achieve greater N_2 fixation and higher yields under these conditions. For example, Adu-Gyamfi et al. (1999) concluded that legumes for the low fertility regions of the semi-arid tropics "should have deep rooting systems to pump nutrients from the deep soil layers, moderately high nitrogen fixing ability, should be adapted to areas of poor rainfall, contribute a substantial amount of biomass to soil prior to harvest in the roots and through leaf fall, and have ability to grow under low P status soils".

Phosphorus may be a critical constraint of legumes under low-nutrient environments because there is a substantial need for P in the N_2 fixation process. One clear manifestation of this may be the common observation that application of P on a mixed grass-legume pasture will enhance the legume growth relative to the grass. The high requirement for P in legumes is consistent with the involvement of P in the high rates of energy transfer that must take place in the nodule. For example, Figure 3 illustrates the sensitivity of N_2 fixation in three common bean cultivars to varying levels of P.

Sa and Israel (1995) also found that N_2 fixation in soybean was very sensitive to low available P. They further indicated that P deficiency might be directly
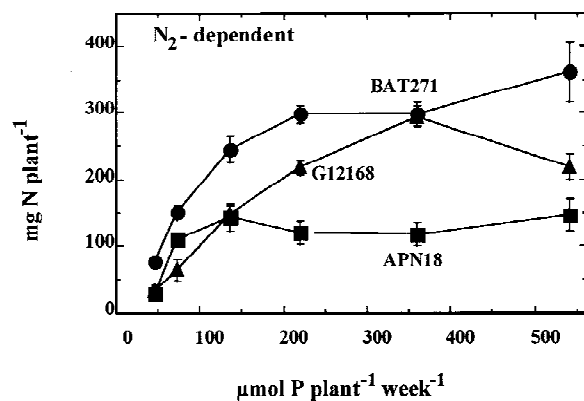
Figure 3. Nitrogen fixed in three common bean genotypes grown for 50 days in a N-free nutrient solution under various weekly supplies of P. Up to 200 μM P plant^{-1} plants absorbed all P supplied and could be compared on the basis of a similar P uptake. From Vadez et al. (submitted).

involved in the active loading of ureides, which are the compounds transported from soybean nodules, into the xylem for transport to the plant shoot. However, a contributing factor to the results of Sa and Israel (1995) was that the P deficiency severely decreased nodule mass so that the ability to produce ureide was drastically decreased. In any case, the high nodule ureide concentration observed under P deficiency might be explained by an increased specific nodule activity, which can be increased under P deficiency as a tolerance mechanism developed by certain bean cultivars (Vadez et al., 1997) or *Acacia mangium* (Ribet and Drevon, 1996; Sun et al., 1992).

Nodule P concentration is usually higher than in other plant tissues (Jakobsen, 1985; O'Hara et al., 1988; Robson et al., 1987). High P levels in nodules might be associated with the abundance of membranes, bacteroid, peri-bacteroid and other membranes, which are 20 times more abundant in infected than in non-infected cells of the nodule (Bergersen, 1994). Also, the energy charge is less affected in the bacteroid fraction of nodules than in the plant fraction of nodules (Sa and Israel, 1991). This might indicate that P is more tightly sequestered in infected than in non-infected cells of nodules. A consequence is that nodule P concentration is less affected by P deficiency (Adu-Gyamfi et al., 1989; Vadez et al., 1997). P deficiency seems to be readily accommodated by nodules of soybean (Ribet and Drevon, 1995) and common bean (Vadez et al., 1997) by decreasing the size of individual nodules.

A deficiency in soil P is not likely to be a hindrance to rhizobium development and the ability to initiate nodulation. Beck and Munns (1984) showed that rhizobium cells are able to take up P at very low solution concentrations (0.06–0.5 μM). In addition, the bacteria were able to accumulate excess P by forming polyphosphate granules that could sustain 3–4 subsequent generations of bacteria.

An important consideration for introducing legumes to a low-P environment, is the ability to sustain N_2 fixation activity when P levels are low. A key issue might be the distribution of P between the different organs of symbiotic plants under P deficiency. Those plants in which the allocation of P to nodules is a high priority are more likely to be able to sustain high N_2 fixation rates. Vadez et al. (1997, 1999) identified common bean cultivars that showed a capacity to fix 2–3 times more N at low, similar P-uptake levels (Figure 3). This was mainly explained by differences in the pattern of allocation of P in plants, with a higher allocation of P to nodules in tolerant genotypes. A similar result was found in superior fixing lines under P deficiency of mungbean (Gunawardena et al., 1992) where nearly 20% of total plant P was allocated to nodules. It has also been observed that a genotype in each species of *Acacia mangium* or pigeonpea is tolerant to P deficiency and appears to maintain a high level nodulation under low P conditions (Adu-Gyamfi et al., 1989; Sun et al., 1992). A comparison between common bean and cowpea receiving similar deficient P showed higher fixing capacity in cowpea than in common bean, which was correlated to a higher percentage of total plant P allocated to nodules in cowpea (Vadez and Gomez, pers. com.). Again, an advantage might be conferred to long-season cultivars, where competition for P between reproducing organs and nodules occur later in the cycle and allow for enhanced N_2 fixation. Indeed, Vadez et al. (1999) found in a screening of 48 genotypes receiving similar P that higher fixing lines were mostly long-season cultivars.

Conclusions

Nitrogen and phosphorus are essential components of virtually all the key molecules and tissues that make plant life possible. Deficiencies in either element result in quantitative decreases in the physiological activity of the crop. Leaf area development is one of the more sensitive responses of crop growth to N and P deficiencies. Photosynthesis rates are also decreased in

response to severe limitations of N and P accumulation in the plant.

Some alterations in plant traits may be possible to ameliorate to a degree the large losses in plant activity that can occur with inadequate N and P. The N limitation in mass accumulation by C_3 crop species under low-N environments could be greatly decreased if the C_4 photosynthetic system could be introduced into C_3 species. This is a very complicated modification of the plant and certainly remains a speculative option. The P limitation for respiration can be minimized by increased utilization of enzymes that do not require P. This is an expensive alternative in terms of energy use in the plant, but it may be a good alternative under low-nutrient environments. Again, this approach is speculative and evidence of genotypic variation first needs to be gathered.

In the final analysis, however, increased grain yield requires increased N and P accumulation to synthesize the constituents of the developing seeds. Seeds have a clear requirement for both elements and it is not possible to increase crop yields without additional amounts of both elements to support seed growth. This perspective indicates that increased accumulation of N and P are obligate requirements to increase crop yield in low-nutrient environments.

Unfortunately, options for increasing nutrient uptake from the soil are somewhat limited. Roots have the ability to readily accumulate soluble N and P from the soil. Since soluble forms of N are highly mobile in the soil, there is no indication that altered root morphology would improve uptake. Phosphorus, on the other hand, is highly immobile and increased surface area in the rhizosphere for P uptake may well be useful for some crops. Specific modifications in length of root hairs or more extensive symbiosis with mycorrhiza might prove beneficial. Excretions from roots to stimulate the conversion of P from unavailable forms in the soil to more available forms offer an intriguing possibility.

One striking possibility to increase crop yields in low-nutrient environments is the increased use of legumes. Legumes offer the possibility of introducing N to the cropping system through symbiotic N_2 fixation. This fixed nitrogen can be used directly for seed production in grain legumes, or for soil enrichment by the incorporation into the soil of legume plant material. Legumes, however, still require P to sustain physiological activity and to grow tissues, including seeds. A major focus of research for low-nutrient environments may well be on the economy of P availability and accumulation by legumes. Approaches to enhance recovery of soil P by legumes will likely be a high priority to increase productivity of whole cropping systems in low-nutrient environments.

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